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Original Articles

HLA antigens and asthma in Greeks

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HLA-A and -B antigens were determined in a group of 76 Greek asthmatic patients: 35 children (1·5–15 years) and 41 adults (18–73 years). The results were compared to those of 400 healthy unrelated controls from the same population. The standard NIH lymphocytotoxicity test was applied. When all 76 patients were compared to the controls, a statistically significant lower frequency of HLA-B5 and -B35 antigens was noted. When adults were analysed alone, an increased frequency of HLA-B8 was found. On the other hand, in the asthmatic children sub-group, the HLA-A10 antigen was significantly higher and the HLA-B5 was significantly lower than in the controls. These data imply that different HLA antigens may be involved in the pathogenesis of several clinical forms of asthma and that, in order to study the role of immunogenetic factor(s) in the pathogenesis of this disease, more adequate grouping criteria are needed.

Introduction

The hereditary-immunogenetic background underlying asthma pathogenesis is generally accepted. However, the role of a particular HLA type(s) to which this disease is related remains unclear (1). Thorsby *et al.* (2) reported an increased frequency of HLA-B40 in asthmatics and this finding was supported by the study of Bruce *et al.* (3). Although both results were statistically insignificant, a Chinese group also found a significant association between asthma and HLA-B61, a split product of HLA-B40 in a Manchurian population (4). Morris *et al.* suggested (initially) an increased frequency of A1 and B8 in extrinsic asthmatics and a decreased frequency of HLA-B12 in intrinsic asthmatics (5). However, the same author later denied these initial observations and found increased frequency of HLA-B12 in extrinsic asthmatics and decreased frequency of HLA-A3, -B7 and -DR2 in intrinsic asthmatics (6). After studying 10 family groups, Brady claimed no association between HLA and asthma (7).

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†Dr Jason Zervas died while this paper was in preparation. The authors wish to dedicate this work to his memory, as among his other scientific and educational contributions, he has been a pioneer of the HLA studies in Greece.

As far as the Greek population is concerned, the relevant information is limited. This paper reports a study of the HLA types in a sample of Greek asthmatics.

Materials and Methods

HLA-A and -B antigens were determined in a group of 76 Greek asthmatic patients: 35 children (1·5–15 years) and 41 adults (18–73 years). The diagnosis of asthma was based on: (1) a history of variable wheeze, shortness of breath, chest tightness or cough; or (2) a documented spontaneous or β_2-agonist-induced airflow reversibility, recorded as a change of 20% on forced expiratory volume in 1 s (FEV_1), or peak expiratory flow rate (PEFR) and/or airway hyper-responsiveness as measured by metacholine in an inhalation test. In the asthmatics, the provocation concentration of metacholine to cause a 20% fall in FEV_1 (PC_{20}) was <1 mg ml^{-1}. All patients were classified as suffering from extrinsic or intrinsic asthma. Patients with atopy were considered as having extrinsic asthma. Atopy was defined as the presence of a wheal greater than 3 mm, as compared with the diluent control, in response to skin prick testing to at least one of the following common aeroallergens: grasses, grasses/cereals, trees I, trees II, olive, timothy, dahlia, chrysanthemum,

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Table 1 Frequency distribution of HLA-A and -B antigens in the whole group of 76 Greek asthmatics and in 400 unrelated controls from the same population

HLA antigens	Patients (n=76)		Controls (n=400)		Statistical difference	Relative risk
	No	%	No	%		
A1	33	43·4	103	25·7		
A2	40	52·6	207	51·2		
A3	17	22·4	99	24·7		
A9	11	14·5	130	32·5		
A10	27	35·5	55	13·7	Pc=0·009	3·45
A11	10	13·3	34	8·5		
A28	6	7·8	40	10		
B5	6	7·8	122	30·5	Pc=0·00003	0·19
B7	13	17·1	57	14·2		
B8	27	35·5	55	13·7		
B12	26	34·2	74	18·5		
B13	5	6·5	40	10		
B14	15	19·7	37	9·2		
B15	9	11·8	30	7·5		
B17	9	11·8	39	9·7		
B27	13	17·1	27	6·7		
B35	3	3·9	69	17·2	Pc=0·0065	0·19
BW40	3	3·9	27	6·7		

Pc, *P*-value corrected for comparisons made.

acacia, weeds, wheat flour, glazkrant, house dust, mite I-D farine, mite II-D pteronyssimus, dog, cat, cladosporium, herbarum, *Alternaria tenuis, Aspergillus fumigatus* (Allergopharma, Joachim Gazer KG Hambourg). Patients without hypersensitivity to aeroallergens or to other known external causal factors were considered as having intrinsic asthma. Thirty children and 20 adults were classified as extrinsic asthma patients, whereas the remaining (five children and 21 adults) were classified as intrinsic asthma patients. In all adults tested, the age of onset of symptoms was at least the 17th year of age; accordingly, adults with asthma lasting from childhood were not typed. Also, cases with unclear medical history were not typed.

For the HLA typing, the standard NIH microlymphocytotoxicity test was applied. Fifty-seven HLA antisera were utilized, testing for a total of 18 HLA-A and -B antigens (7 and 11, respectively). Due to limited availability of some HLA antisera, only those testing for the main specificities were used; accordingly, HLA antisera testing for several split products were not applied. The results were compared to those of 400 healthy unrelated Greek controls (blood donors aged 20–55 years). As HLA frequencies do not vary with age in the general population (8), this control group is considered as matched with patients of every age.

The estimated frequency of HLA homozygotes in the samples (patients–controls) was at the order of 5% which is in agreement with the current data for Caucasian populations, including Greeks (9,10).

For statistical evaluation, Fischer's exact test was applied and the P values were corrected for comparisons made (Pc). Criterion of significance was considered to be Pc less than 0·01. Relative risks were also calculated (9).

Results

Table 1 depicts the frequency distribution of HLA -A and -B antigens in the total group of 76 patients and in 400 controls in numbers and percentages. As can be seen, HLA-B5 and -B35 antigens were found in a frequency which was significantly lower than that of the controls (7·8 *vs.* 30·5% and 3·9 *vs.* 17·2%, respectively). When the adults were analysed alone (Table 2), a significantly higher frequency of HLA-B8 antigen (46·3 *vs.* 13·7%) was found. When the childrens sub-group (alone) was compared to the controls (Table 3), the HLA-A10 antigen was found to be significantly increased (45·7% *vs.* 13·7%).

Table 2 Frequency distribution of HLA-A and -B antigens in 41 asthmatic Greek adults and in 400 unrelated controls from the same population

HLA antigens	Patients (n=41)		Controls (n=400)		Statistical difference	Relative risk
	No	%	No	%		
A1	16	39·02	103	25·7		
A2	26	63·4	207	51·2		
A3	12	29·3	99	24·7		
A9	6	14·63	130	32·5		
A10	11	26·82	55	13·7		
A11	6	14·63	34	8·5		
A28	0	0	40	10		
B5	6	14·63	122	30·5		
B7	7	17·07	57	14·2		
B8	19	46·4	55	13·7	Pc=0·0019	5·4
B12	12	29·26	74	18·5		
B13	2	4·87	40	10		
B14	7	17	37	9·2		
B15	5	12·19	30	7·5		
B17	4	9·75	39	9·7		
B27	5	12·19	27	6·7		
B35	1	2·43	69	17·2		
BW40	1	2·43	27	6·7		

Pc, *P*-value corrected for comparisons made.

Table 3 Frequency distribution of HLA-A and -B antigens in 35 asthmatic Greek children and in 400 unrelated controls from the same population

HLA antigens	Patients (n=35)		Controls (n=400)		Statistical difference	Relative risk
	No	%	No	%		
A1	17	48·6	103	25·7		
A2	14	40	207	51·2		
A3	5	14·3	99	24·7		
A9	5	14·3	130	32·5		
A10	16	45·7	55	13·7	Pc=0·0068	6·69
A11	4	11·4	34	8·5		
A28	6	17·1	40	10		
B5	0	0	122	30·5	Pc=0·0003	
B7	6	17·1	57	14·2		
B8	8	22·8	55	13·7		
B12	14	40	74	18·5		
B13	3	8·6	40	10		
B14	8	22·9	37	9·2		
B15	4	11·4	30	7·5		
B17	5	14·3	39	9·7		
B27	8	22·8	27	6·7		
B35	2	5·7	69	17·2		
BW40	2	5·7	27	6·7		

Pc, *P*-value corrected for comparisons made.

Interestingly, the HLA-B5 antigen was absent in patients of this age group (0 *vs.* 30·5%).

Discussion

Asthma seems to be an heterogeneous entity and its classification into several forms faces considerable difficulties related to pathogenesis, clinical presentation, natural course and prognosis. Grouping patients according to the age of onset is quite an indisputable way of classifying the disease. On the other hand, classification into extrinsic and intrinsic asthma is sometimes difficult and, to a significant degree, overlapping. The role of histocompatibility antigens in the pathogenesis of asthma remains divergent (1). Several authors have suggested an increased frequency of several HLA types in patients of different population groups and different forms of the disease, which other authors failed to confirm. It is worth noting that occasionally the same group of investigators, after a more extensive study, do not confirm the results of their own preliminary work (5,6). As the pattern of linkage disequilibrium may vary considerably among several populations, it is obvious that different antigens may be associated with the same disease in different racial groups and populations. On this point, it is worth mentioning the ethnic homogenicity of the population of Greece.

It is thus evident that a different pattern of the distribution of HLA-A and -B antigens was observed in the present study. In the children's group, a high frequency of HLA-A10 and a lower frequency of HLA-B5 was found. On the other hand, in the adult group, a higher frequency of HLA-B8 was detected; the HLA-A1 antigen, which is in linkage disequilibrium with HLA-B8 in most Caucasian populations including Greeks (10), was not found to be increased in this sub-group.

In the interest of completeness, it should be added that when extrinsic asthmatic children alone were analysed, no significant differences in the frequency distribution of all the above mentioned antigens were detected. However, in the adult group, the higher frequency of HLA-B8 was observed only in cases of extrinsic type (data not shown).

This study provides some evidence for a possible association between a particular HLA type(s) and bronchial asthma when the subjects tested are grouped according to the age of onset of the disease. These results support the concept of heterogenicity in immunogenetic factors implicated in the pathogenesis of this disease, and provide further evidence that in the search of the role of the HLA genes in a particular disease entity, the homogenicity of the subjects tested should be secured as much as possible. To the best of the authors' knowledge, the present study is the first one of this type in Greek asthmatics. Further studies on this topic should be encouraged; studies on HLA-Class II types are also needed.

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